

REMARKS

Applicants request reconsideration of the outstanding rejections and objections in view of the amendments and comments set forth herein.

Claims 51 and 56 have been amended to include an inducible promoter and to remove "or plastid" in order to separately claim transforming plastids with β -1,4-endoglucanase, the subject matter of which can now be found in new claims 74 to 80. Support for inducible promoter can be found on page 11, line 15 to page 12, line 7, as well as, various places through out the specification.

Claims 52, 54, 60 and 62 have been canceled in order to write the subject matter of those claims in independent form. The subject matter for these claims can now be found in new claims 81 to 86.

Claim 55 has amended to remove "or plastid" in order to separately claim transforming plastids with β -1,4-endoglucanase, the subject matter of which can now be found in new claims 74 to 80..

Claims 57 and 64 have been amended to remove "or plastid" in order to separately claim transforming plastids with β -1,4-endoglucanase. These new claims can now be found in new claims 74 to 80. The phrase "and wherein the targeting sequence will target the microbial β -1,4-endoglucanase to an organelle or cell compartment where the microbial β -1,4-endoglucanase will not be able to degrade cellulose" has been added to claims 57 and 64 to clarify the targeting sequence. Support for this amendment is on page 6, line 29 to page 7, line 4, on page 24, lines 9 to 11, and in the abstract. Specifically, page 7, lines 3-4 contains the phrase "they will not be able to degrade cellulose". "They" in this instance refers to cellulases. The phrase "organelle or cell compartment" can be found specifically on page 24, line 11.

Claim 59 has been amended to remove "a wound inducible or chemically inducible promoter" and replace it with "an inducible promoter". Support for inducible promoter can be found on page 11, line 15 to page 12, line 7, as well as, various places through out the specification.

Claims 63 and 65 have been amended to fix a typographical error in the spelling of mitochondria and to remove "apoplast and extracellular secretion from aleurone cells". Applicants wish to thank the Examiner for pointing out this spelling error.

New claims 66 and 67, which depend from claim 51, and claims 70 and 71, which depend from claim 57, have been added to include when the microbial β -1,4-endoglucanase is from either a cellulolytic bacterium or a filamentous fungus. Support for these claims can be found in the specification on the first paragraph of page 4; page 3, lines 17-20; page 10, lines 8-13; and page 14, line 28 to page 15, line 15, especially page 15, lines 13-15.

New claims 68 and 69, which depend from claim 51, and claims 72 and 73, which depend from claim 59, have been added to specify when the promoter is a wound inducible or chemical inducible promoter. Support for these claims can be found in numerous places throughout the specification but especially on page 11, line 15 to page 12, line 7 and previously presented claims 55 and 59.

New claims 74 to 80 have been added to separately claim transformation of the chloroplast with β -1,4-endoglucanase. Support for these new claims can be found in the previously presented claims 57 to 65.

New claims 81 to 86 have been added to separately claim transgenic plants comprising a β -1,4-endoglucanase from a *Thermomonospora* bacterium or more specifically from a *T. fusca* bacterium. Support for these new claims can be found in previously presented claims 52, 54, 60 and 62.

Claims 51, 53, 55-59, 61, 63-86 are currently pending.

35 U.S.C. § 112, first paragraph, Written Description

The Office Action mailed June 26, 2007 rejects claims 51, 53, 57, 61 and 63 under 35 U.S.C. § 112, first paragraph. The Office Action suggests that the "specification fails to describe the structural features that confer thermostability on a microbial β -1,4-endoglucanase". Further, because "the genus is highly variant" and the "the disclosure fails to describe the common attributes that identify members of the genus" then the "disclosed species are insufficient to describe the claimed genus".

Applicants respectfully traverse. Microbial β -1,4-endoglucanases, including thermotolerant enzymes, were well known and studied at the time of filing of the present application. To fulfill the written description requirement it is not necessary in a patent application to describe information and facts known to those skilled in the art or list every possible endoglucanase known at the time of the invention. "A patent need not teach, and preferably omits, what is well known in the art." *Hybridtech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). Even in a genus with substantial variation, disclosing a representative number of species either implicitly or explicitly will meet the written description requirement (See page 8 of the Written Description Guidelines for the Written Description Original Claims Decision Tree).

Applicants submit that a representative number of species of thermotolerant β -1,4-endoglucanase was known in the art at the time of filing of the present application. In addition, it was also recognized at the time of filing that new thermostable β -1,4-endoglucanase could be found by isolation from thermophilic organisms or non-thermotolerant enzymes could be engineered to have improved thermotolerance. (See page 30, section 1.5.2 entitled "Sources of thermostable enzymes:" of Gilbert's Ph.D. thesis on "Production and Characterization of Cellulases and Xylanases from the Thermophilic Ascomycete *Theilavia terrestris* 255B", December 1992.)

As Applicants have discussed previously, Gilkes, *et. al.* and Henrissant, *et. al.* describe in detail the sequence, structure and function of microbial endoglucanases. Gilkes, *et. al.* disclose a number of endoglucanases, including a number of thermotolerant endoglucanases. See Table 5 in Gilkes, *et. al.* (1991), Microbiological Reviews, Vo. 55, No. 2, pp. 303-315. In table 5, the following entries are thermostable enzymes having endoglucanase activity: A3, A4, A12, A15, A17, A18, and B2. This represents thermostable enzymes from a variety of organisms including *Bacillus* sp.; *Calocellum saccharolyticum*; *Clostridium thermocellum*; and *Microbispora bispora*. Previously cited Jung *et. al.* and Lao, *et. al.* describe several thermotolerant endoglucanases and in particular endoglucanases from *T. fusca*.

In addition, Applicants would like to draw the attention of the Examiner to the following new references, all of which are now included in the Supplemental Information Disclosure Statement submitted herewith. In a review of cellulases of bacterial origin, Robson and Chambliss (1989) *Enzyme Microbiology Technology* (11) pp. 626-644, describe β -1,4-endoglucanases from thermophilic or mesophilic bacteria, including enzymes of the bacterial genera *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermospora*, and *Acetivibrio*. Collings, et. al. (1988) *Microbios* (56) pp. 131-147, describe β -1,4-endoglucanase activity from the filtrate of a number of thermophilic, thermotolerant and mesophilic fungi including *A. niger*, *A. fumigatus*, *G. emersonii*, *P. janthinellum*, *P. funiculosum*, *T. viride* and *P. ochrochloron*. (In particular, see page 136, second paragraph and figure 4 on page 141). Thermotolerant β -1,4-endoglucanases were purified from the thermophilic fungus *allescheria terrestris* by Kvesitadze, et. al. as described in 1994 in *Microbios* (80) pp. 115-123. Baker, et. al., (1994) *Applied Biochemistry and Biotechnology*, (45/46), pp. 245-256, describe a new thermostable endoglucanase from *Acidothermus cellulolyticus*. Romaniec et. al. (1987) *Journal of General Microbiology* (133) pp. 1297-1307, describe 13 new *C. thermocellum* genes with endoglucanase activity. A new thermophilic β -1,4-endoglucanase was isolated from *Clostridium thermocopriae*, sp. Nov. JT3-3 by Jin and Toda in the 1989 *Journal of Fermentation and Bioengineering*, Vo. 67, No. 1, pp. 8-13. Presutti, et. al. (1991) *Journal of Biotechnology* (17) pp. 177-188 describe three *Thermomonospora curvata* thermophilic endoglucanase genes.

Further, it was recognized that the thermostability of enzymes and in particular cellulases could be increased by protein engineering. See page 30 of Gilbert, *supra*. Nosoh and Sekiguchi, (1990) *Tibtech* (8) pp. 16-20 describe how site-directed mutagenesis can be used to produce more thermostable proteins. See also, Goodenough and Jenkins (1991) *Bio Chem. Soc. Trans.* (19) pp. 655-662 (describing the contribution of hydrogen bonding, electrostatic interactions and disulphide bridges make to thermostability); Goodenough (1995) *Molecular Biotechnology* (4) pp. 151-166 (describing how the "present state of knowledge allows proteins to be mutated to increase or decrease stability"); Menedez-Arias and Argos (1989) *Journal of Molecular Biology* (206) pp. 397-406 (describing how to engineer thermostable proteins based on

three-dimensional structure); Matthews, *et al.* (1987) Proc. Natl. Acad. Sci. USA (84) pp. 6663-6667 (describing how the thermostability of a "protein can be increased by selected amino acid substitutions that decrease the configurational entropy of unfolding"); Matsumura, *et al.* (1986) Nature (323) pp. 356-359 (describing the "cumulative effect of intragenic amino-acid replacements on the thermostability of a protein"); Imanaka, *et al.* (1986) Nature (324) pp. 695-697 (describing how thermostability of an enzyme can be enhanced by a single amino acid substitution); Arnold (1993) FASEB (7) pp. 744-749 (describing random mutagenesis for increasing thermostability of enzymes); Politz, *et al.* (1993) Eur. J. Biochem. (216) pp. 829-834 (describing hybrid (1-3,1-4)- β -glucanases with increased thermostability that were further enhanced by site-directed mutagenesis); PCT Publication No. WO 90/09436 (describing how to make hybrid thermostable (1-3, 1-4)- β -glucanases) and US Patent No. 6,277,615, filed Jan. 11, 1996, which was a Continuation-in-Part of application No. PCT/AU94/00377, filed on July 6, 1994 (describing a (1-3, 1-4)- β -glucanase having been modified for increased thermostability by site-directed mutagenesis).

To facilitate the engineering of proteins for thermostability, knowledge of the three dimensional structure of the protein is helpful. Knowledge of the three dimensional structure of β -1,4-endoglucanase was well known in the art at the time of filing. The three-dimensional structure of the thermostable endoglucanase CelD from *Clostridium thermocellum* was well known at the time of filing. Please see, Juy, *et al.* (1992) Nature, Vol 357, pp. 89-91 and Chitarra, *et al.* (1995) J. Mol. Biol. (248) pp. 225-232. See also, Davies, *et al.* (1993) Nature (365) pp. 362-364 (describing the structure and function of endoglucanase V); Spezio, *et al.* (1993) Biochemistry (32) pp. 9906-9916 (describing the crystal structure of E2 from *T. fusca*); Dominguez, *et al.* (1994) PROTEIN: Structure, Function and Genetics (19) pp. 158-160 (describing the two crystal forms of *C. thermocellum* endoglucanase CelC); Davies, *et al.* (1992) J. Mol. Biol. (228) pp. 970-972 (describing the crystallization and preliminary X-ray analysis of EG1 from *Humicola insolens*); Sakon, *et al.* (1996) Biochemistry (35) pp. 10648-10660 (describing the crystal structure of the thermostable family 5 endocellulase E1 from *Acidothermus cellulolyticus*).

In conclusion, not only were numerous thermophilic endoglucanases known and well studied, but it was also known how to find additional thermotolerant β -1,4-endoglucanase from thermotolerant microorganisms and how to engineer a non-thermostable enzyme to improve its thermostability. Therefore, Applicants submit that a representative number of species of thermotolerant β -1,4-endoglucanases was known in the art at the time of filing. Applicants respectfully request withdrawal of the current rejection under 35 U.S.C. § 112, written description.

35 U.S.C. § 112, first paragraph, Enablement

The present Office Action rejects claims 51, 53, and 57-65 under 35 U.S.C. § 112, first paragraph because "(t)he specification only teaches one source of thermostable microbial β -1,4-endoglucanases, those from *T. fusca*, and does not teach how to make other thermostable microbial β -1,4-endoglucanase."

Applicants respectfully traverse. As stated previously, (a) patent need not teach, and preferably omits, what is well known in the art." *Hybridtech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986), *et. denied*, 480 U.S. 947 (1987).

As discussed in the Written Description section above, thermotolerant microbial β -1,4-endoglucanases were well known in the art, including the structure of thermophilic enzymes as exemplified by the disclosure in Gilkes, *et. al.*, Henrissant *et. al.*, Jung, *et. al.*, Juy *et. al.*, Lay *et. al.*, and the additional references cited above. In addition, the biochemical properties, functional structure, methods and assays for finding new β -1,4-endoglucanase and methods of engineering thermostable enzymes were well known in the art as described in the references cited above.

Claims 63 and 65 stand rejected in the current Office Action because "(t)he specification teaches no such targeting sequences that function on a protein that is made within the chloroplast and that targets the protein out of the chloroplast into another organelle". Applicants have amended claims 51 and 57 to remove "or plastid".

Applicants have added new claims 74 to 80 to describe a transgenic plant expressing an endoglucanase under the control of an inducible promoter in a plastid genome.

In light of the remarks and claim amendments above, Applicants respectfully request withdrawal of the current rejections under 35 U.S.C. § 112, enablement.

35 U.S.C. § 103, Obviousness

In the present Office Action claims 51-54, 56-58, 60-65 stand rejected over Van Ooyen, *et. al.* in view of Lao, *et. al.*. It is suggested by the present Office Action that it would be obvious to modify the plants and seeds expressing amylase taught by Van Ooyen, *et. al.* to express the endoglucanase enzymes taught by Lao, *et. al.* It is suggested by the present Office Action that motivation to combine the two references is provided in Lao *et. al.* because microbial β -1,4-endoglucanase were available and the selection of one microbial β -1,4-endoglucanase "over another is an obvious design choice and optimization of experimental parameters" and by Van Ooyen *et. al.* because Van Ooyen *et. al.* suggests expression of microbial β -1,4-endoglucanase in plants and discloses targeting of a protein to various cellular compartments.

Applicants respectfully traverse. As stated in *Ex parte* Catan, the "functional approach" is "to be taken in cases where the claimed invention is a prior art structure altered by substituting one element in the structure for another know element". *Ex parte* Catan, precedential opinion of the United States Patent and Trademark Office before the Board of Patent Appeals and Interferences, Appeal 2007-0820, July 3, 2007, p. 11, citing, *KSR*, 127 S.Ct. at 1734, 82 USPQ2d at 1391 and discussing *United States v. Adams*, 383 U.S. 39, 40, 148 USPQ 479, 480 (1966). The Board in *Ex parte* Catan went on to say that "the *Adams* Court found the combination at issue *not* obvious to those skilled in the art because, although the elements were known in the prior art, they worked together in an *unexpected* manner". *Id.* at p. 11.

Targeting transgenically produced cellulases to organelles or cellular compartments can prevent the cellulase from degrading cellulose and thus harming the plant. See the bottom of page 6 to the top of page 7 of the specification. Van Ooyen

does not anticipate or teach one how to prevent cellulase from being toxic to a plant. In fact, Van Ooyen *et. al.* teaches away from targeting cellulases to an organelle or cellular compartment not containing an enzyme's substrate by suggesting that one would target "the expressed enzyme to a predetermined locus in order to have better access of the enzyme to its substrate." See column 6, lines 53 to 58 of US patent no. 5,705,375.

Applicants would like to draw the attention of the Examiner to US Patent No. 7,102,057, which is included in the enclosed Supplemental Information Disclosure Statement. Example 13, starting near the end of column 56 on line 57, describes transformation and expression of thermotolerant amylase fused to a waxy amyloplast targeting peptide (pNOV4029) or an amylase/waxy fusion protein fused to a waxy amyloplast targeting peptide (pNOV4031). Expression of the amyloplast targeted amylase or amylase fusion protein in the endosperm of a corn seed causes the amylase enzyme to have better access to its substrate, i.e. the starch containing amyloplast. As can be seen in Example 13, targeting the enzyme to its substrate resulted in shriveled seed containing little to no starch. Seed containing little to no starch is not viable. One of skill in the art using the teaching of Van Ooyen and targeting amylase to ensure access of the enzyme to the substrate would result in harm to the plant or an unexpected result. Therefore, given the teaching of Van Ooyen, even in light of Lao, *et. al.*, targeting cellulase away from its substrate to reduce harm to a plant would be an unexpected result.

Applicants respectfully submit that the Office has not met its burden of establishing a *prima facie* case of obviousness over Van Ooyen *et. al.* in light of Lao, *et. al.* Specifically, Applicants submit that Van Ooyen teaches away from targeting an enzyme away from its substrate and that reducing toxicity of a plant expressed cellulase by targeting the enzyme to an organelle or cellular compartment not containing the substrate would be an unexpected result. Applicants request reconsideration and withdrawal of the obviousness rejection.

The current Office Action suggests that Van Ooyen *et. al.* discloses an inducible (patatin) promoter (page 6, second full paragraph of the present Office Action). As previously discussed and agreed to, the patatin promoter as described in Van Ooyen, *et.*

al. in column 6, lines 32 to 34, is not an "inducible" promoter but rather is a tuber specific (or tissue specific) promoter.

CONCLUSION

Applicants believe that the above remarks and current claims overcome the rejections to the claims. Reconsideration of the application and allowance of all pending claims is earnestly solicited.

Should the Examiner wish to discuss any of the above in greater detail or deem that further amendments should be made to improve the form of the claims, the Examiner is invited to telephone the undersigned at the Examiner's convenience.

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